

Low-molecular-mass proteins in cyanobacterial photosystem II: identification of *psbH* and *psbK* gene products by N-terminal sequencing

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The O₂-evolving photosystem II core complex was isolated from a thermophilic cyanobacterium, *Synechococcus vulcanus* Copeland. Analysis by SDS-polyacrylamide gel electrophoresis revealed that the complex contained at least seven low-molecular-mass proteins in addition to the well characterized CP47 apoprotein, CP43 apoprotein, 33 kDa extrinsic protein, D1 protein, D2 protein and large subunit of cytochrome *b*-559. The separation profiles of these low-molecular-mass proteins were very similar between cyanobacterial and higher plant PS II. N-terminal sequences of the 6.5 kDa and 3.9 kDa proteins of cyanobacterial core complex were determined after blotting to a polyvinylidene difluoride membrane. The sequence of the 6.5 kDa protein showed high homology with an internal sequence of plant *psbH* gene product, so-called 10 kDa phosphoprotein, but did not conserve the Thr residue which is specifically phosphorylated in plants. The sequence of the 3.9 kDa protein corresponded to the K protein of higher plants (mature form of *psbK* gene product). These results indicate that the products of both *psbH* and *psbK* genes are present in cyanobacterial PS II as well as being associated with the O₂-evolving core complex.

Cyanobacterium; Oxygen-evolving core complex; Photosystem II; Gene, *psbH*; Gene, *psbK*; (*Synechococcus vulcanus*)

1. INTRODUCTION

The O₂-evolving PS II core complex, which is devoid of light-harvesting complexes and other peripheral components, has been hypothesized as the structural minimum for PS II functioning including water oxidizing capacity [1–4]. The complex isolated from both cyanobacteria and higher plants commonly consists of CP47 apoprotein, CP43 apoprotein, 33 kDa extrinsic protein, D1 protein, D2 protein and two subunits of cytochrome *b*-559. Besides these, several low-molecular-mass proteins were recently found to be

associated with the complex of higher plants [5,6]. In PS II membrane fragments retaining light-harvesting complexes, some other low-molecular-mass proteins were additionally found. Thus, for a more comprehensive understanding of the structural minimum for functional PS II, it is important to comparatively determine the common PS II components between cyanobacteria and higher plants. However, there are so far no reports describing the low-molecular-mass components of the cyanobacterial complex in comparison with those in higher plants.

In this communication we report several low-molecular-mass components found in the O₂-evolving core complex isolated from a thermophilic cyanobacterium, *Synechococcus vulcanus*. N-terminal sequencing of two of the components clearly demonstrated that they correspond to the products of *psbH* and *psbK* genes of higher plants.

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Abbreviations: Chl, chlorophyll; PS II photosystem II; SDS-PAGE, SDS-polyacrylamide gel electrophoresis

2. MATERIALS AND METHODS

Thylakoids were prepared from a thermophilic cyanobacterium, *Synechococcus vulcanus* Copeland as described previously [7]. The O₂-evolving PS II particle was prepared by solubilizing the thylakoids with lauryldimethylamine *N*-oxide as described in [8]. This PS II particle still retained a significant amount of allophycocyanin, linker and anchor proteins. For further purification, the PS II particle was suspended in 100 mM NaCl, 40 mM Mes-NaOH (pH 6.0) at 0.25 mg Chl/ml, then Triton X-100 was added to a final concentration of 2.5% (w/v). The suspension was incubated for 5 min on ice with gentle stirring and placed onto a DEAE-Toyopearl 650M column (Toso, Japan) equilibrated with 100 mM NaCl, 40 mM Mes-NaOH (pH 6.0) and 0.05% (w/v) Triton X-100. Most of allophycocyanin, linker and anchor proteins and contaminating PS I particles were not adsorbed on the column. After washing out these proteins, a core complex was eluted with 180 mM NaCl containing buffer. The core complex thus obtained evolved oxygen at a rate of 1000–1400 μ mol O₂/mg Chl per h with ferricyanide as electron acceptor at 40°C. The O₂-evolving PS II membrane fragments and core complex of higher plants were prepared from spinach as described in [4].

For resolution of low-molecular-mass proteins, SDS-PAGE with 7.5 M urea and a 16 to 22% (w/v) polyacrylamide gradient was performed as described in [5]. Separated proteins were stained with Coomassie brilliant blue R-250 (Bio-Rad) or transblotted to a polyvinylidene difluoride membrane (Millipore) using a semidry-type electroblotter as described in [9]. Transferred proteins were stained with Amido black 10B (Bio-Rad) and subjected to a protein sequencer (model 477A, Applied Biosystems).

3. RESULTS

Polypeptide compositions of thylakoids, PS II particles and the O₂-evolving core complex are shown in fig.1. Bands of CP47 apoprotein, CP43 apoprotein, 33 kDa extrinsic protein, D1 protein, D2 protein and the large subunit of cytochrome *b*-559 were identified in PS II particles by monospecific antibodies raised against respective spinach proteins (Koike, H., Ikeuchi, M. and Inoue, Y., unpublished). All of these proteins were nearly quantitatively recovered in the core complex, while lipids, allophycocyanins, linker and anchor proteins were mostly removed by DEAE-Toyopearl column chromatography. In the low-molecular-mass region at least seven bands were separately detected in both PS II particles and the core complex in addition to the band of the large subunit of cytochrome *b*-559. The separation profiles were almost identical between the core complex and PS II particles and similar to those of O₂-evolving PS II membrane fragments and the core complex from spinach. It is thus inferred that

these low-molecular-mass proteins are intrinsic components of the cyanobacterial PS II as well, but are not related with the light-harvesting complex. Although accompanied by several comigrating dense bands, all these low-molecular-mass proteins could be detected as faint bands in thylakoid membranes.

Of the low-molecular-mass proteins found in the cyanobacterial PS II core complex, 6.5 kDa and 3.9 kDa bands were well separated from other bands in SDS-PAGE profile. After transblotting to a polyvinylidene difluoride membrane, these bands were cut out and subjected to protein sequencing. N-terminal 20 amino acid signals and 31 amino acid signals were successfully resolved for 6.5 kDa and 3.9 kDa proteins, respectively (figs 2 and 3).

Computer-assisted homology search revealed that the determined sequence of the 6.5 kDa protein was homologous to an internal sequence of the *psbH* gene product, so-called 10 kDa phosphoprotein, of *Chlamydomonas* [10] and higher plants [11–14] (fig.2). Alignment of these sequences indicates that the *Synechococcus* sequence is most homologous to the tobacco sequence but is least homologous to the *Chlamydomonas* sequence, and that an 11 amino acid insert found in *Chlamydomonas* is missing in *Synechococcus* as well as in higher plants. Notably, the N-terminal segment including consensus Ala-Thr-(Gln)-(Thr)- of *Chlamydomonas* and higher plants is missing in the *Synechococcus* protein. This is not due to proteolysis during preparation, since the same 6.5 kDa polypeptide can be detected in PS II particles and thylakoid (fig.1). In contrast to this big difference in N-terminal sequence, the following sequence showed high homology with the corresponding sequences of plants. Based on the reported sequence of the *psbH* gene [11–14], the wheat, tobacco and spinach proteins are predicted to consist of 72 amino acid residues while the liverwort protein is of 73 amino acid residues (note that the first Met residue is lost after translation [9,11]). All of them show very high homology especially in C-terminal side including the single membrane-spanning segment (not shown in fig.2). If we assume that this C-terminal side is conserved in cyanobacteria, the *Synechococcus* protein is predicted to consist of 60 or 61 amino acid residues, and its molecular mass is calculated to be

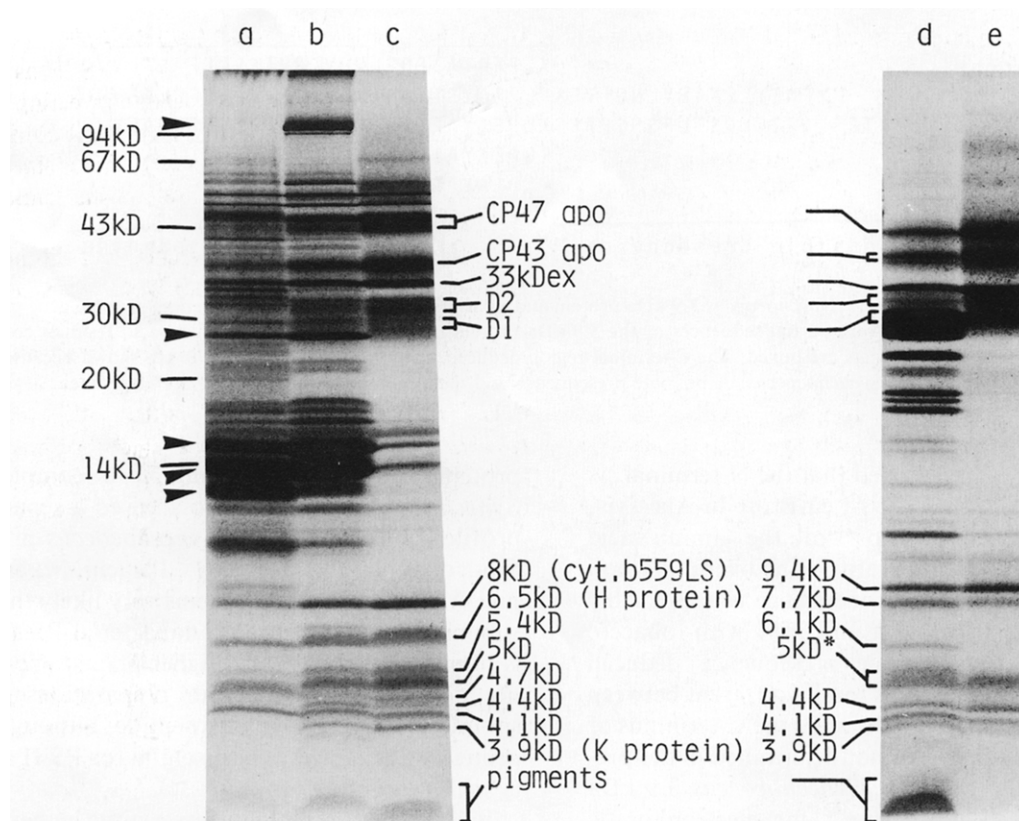


Fig.1. Polypeptide composition of thylakoids (lane a), PS II particles (b) and the core complex (c) from *S. vulcanus*, and PS II membrane fragments (d) and the core complex (e) from spinach. The diffused band of 5 kDa in spinach preparations consisting of at least three different components [9] is indicated by an asterisk. Bands of phycobiliproteins, linker and anchor proteins are indicated by arrowheads. Positions of molecular standards are also shown.

about 6.3 kDa. This estimation is in accordance with the molecular mass determined by SDS-PAGE (6.5 kDa) within error.

The determined sequence of the 3.9 kDa

polypeptide was homologous to a part of the *psbK* gene product of liverwort and tobacco [13,14] and to the N-terminal sequence of the 3.9 kDa protein from spinach and wheat [9,15] (fig.3). Alignment

				ref.
<i>Synechococcus</i>	*	ARR	TWLGDI LRPLNSEY GKV	
<i>Chlamydomonas</i>	ATGT	SKAKPSKVNSDFQEPGLV	TLGLTL LRPLNSEAGKV	10
Liverwort	(M)ATQIIDDTPKTKGKK		SGIGDILK PLNSEY GKV	14
Tobacco	(M)ATQTVENSSRSRGP	RR	TAVGDLLK PLNSEY GKV	13
Wheat	(M)ATQTVEDSSKPRPK	R	TGAGSLLK PLNSEY GKV	9,12
Spinach	(M)ATQTVESSRSRPKP		TTVGALLK PLNSKY GKV	11
	*			

Fig.2. Determined sequence of *Synechococcus* 6.5 kDa protein and its alignment compared with sequences of *psbH* gene products of *Chlamydomonas* and higher plants. Amino acid residues conserved between *Synechococcus* and others are boxed. C-terminal region deduced from *psbH* gene sequence is not shown because it was not determined in *Synechococcus*. The second Thr residue, the phosphorylation site, of *Chlamydomonas* and spinach is indicated by an asterisk. The Met residue for translation initiation, which is missing in the mature protein, is shown in parentheses.

about 5 kDa was detected in O₂-evolving core complex in higher plants [9]. Since this gene sequence is present in *Synechocystis* PCC6803 [21], we can also expect its product in the cyanobacterial PS II core. As shown in fig.1, there are several bands in about the 5 kDa region in the PS II core complex of *Synechococcus*. Some of these will probably correspond to those identified in higher plants.

The *psbH* product of *Chlamydomonas* and higher plants is well known as the 10 kDa phosphoprotein [10,11]. Phosphorylation of the protein is dependent on light illumination, although its physiological function is still uncertain. It is established that the phosphorylation site is the second Thr residue and not the other Thr residues in *Chlamydomonas* and spinach [10,22]. However, this Thr residue and the consensus flanking sequence of *Chlamydomonas* and higher plants were missing in the *Synechococcus* protein. In addition, analysis of protein phosphorylation in *Synechococcus* PCC6301 revealed that no proteins less than 10 kDa were phosphorylated whereas the 18.5 kDa linker protein and the 15 kDa PS II chlorophyll-binding protein were phosphorylated [23]. These results strongly suggest that the *psbH* product is not phosphorylated in *Synechococcus* possibly because of the absence of the second Thr residue. Presumably, it plays some role in PS II other than phosphorylation. Considering this, we propose 'H protein' instead of '10 kDa phosphoprotein' as a general name for the *psbH* gene product. Since the H protein is associated with the O₂-evolving core complex in *Synechococcus* (fig.1) as well as in higher plants [5,24], we may assume that the H protein is essential for functional PS II.

In chloroplast DNA of higher plants, the *psbH* gene is located in an operon in which *psbB* (CP47 apoprotein), *psbH*, *petB* and *petD* (*pet* genes encode components of the cytochrome *b₆/f* complex) are aligned from 5' to 3' [25]. Messenger RNA transcribed from this operon is subject to complex processing [26]. However, little is known about the locations of the *psbH*, *petB* and *petD* genes in the cyanobacterial genome. Although the *psbB* gene and its flanking region of *Synechocystis* PCC6803 were sequenced by Vermaas et al. [27], the *psbH* sequence was not found. Since our results indicate the presence of the *psbH* gene in cyanobacteria, it would be interesting to study whether the *psbH*

gene is co-transcribed with *psbB*, *petB* and *petD* genes or not.

In higher plants, the mature form of the *psbK* gene product, designated K protein, is not associated with the O₂-evolving core complex but with PS II membrane fragments retaining light-harvesting complexes [9,15]. This was interpreted as indicating that the K protein is not essential for the functioning of PS II. However, our finding that it was retained in the O₂-evolving core complex from *Synechococcus* suggests that it is also a common component of PS II. It is unlikely, however, to assume that the K protein functions in connecting light-harvesting complexes with the PS II core, since the light-harvesting complexes are quite different between higher plant and cyanobacterial PS II. A possible role of the K protein, if any, may be to structurally support the PS II assembly.

The K protein of both *Synechococcus* and higher plants contains a single membrane-spanning region on the C-terminal side and several charged residues on the N-terminal side. The presequence deduced from the *psbK* sequence of tobacco and liverwort DNA contains a relatively hydrophobic stretch and an Ala or Gly residue at the C-terminal end. The nature of these presequences resembles those of cytochrome *f* and plastocyanin which are translocated across the thylakoid membrane [28]. This probably indicates that the N-terminal hydrophilic region of K protein protrudes into the lumen space during maturation as a result of processing.

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